Exhibit C

Bioavailability and Bioequivalence:
General Concepts and Overview

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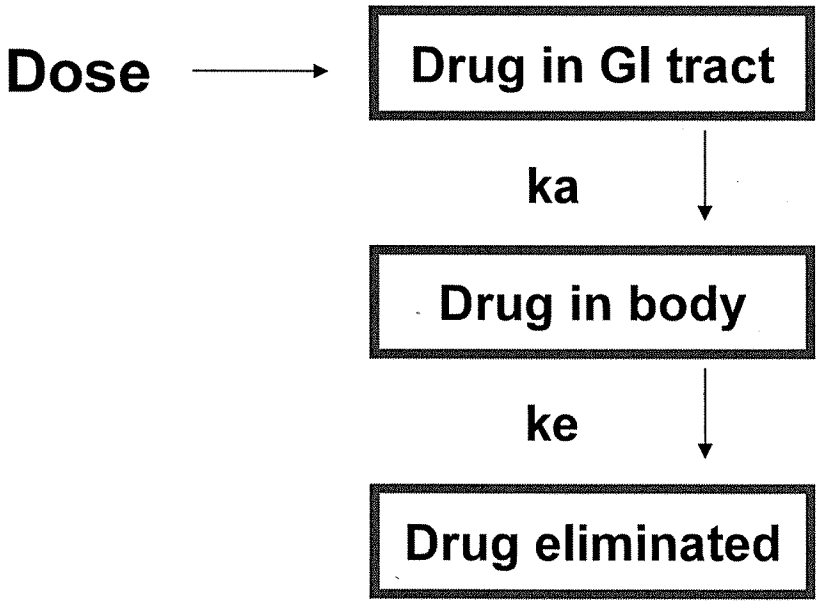
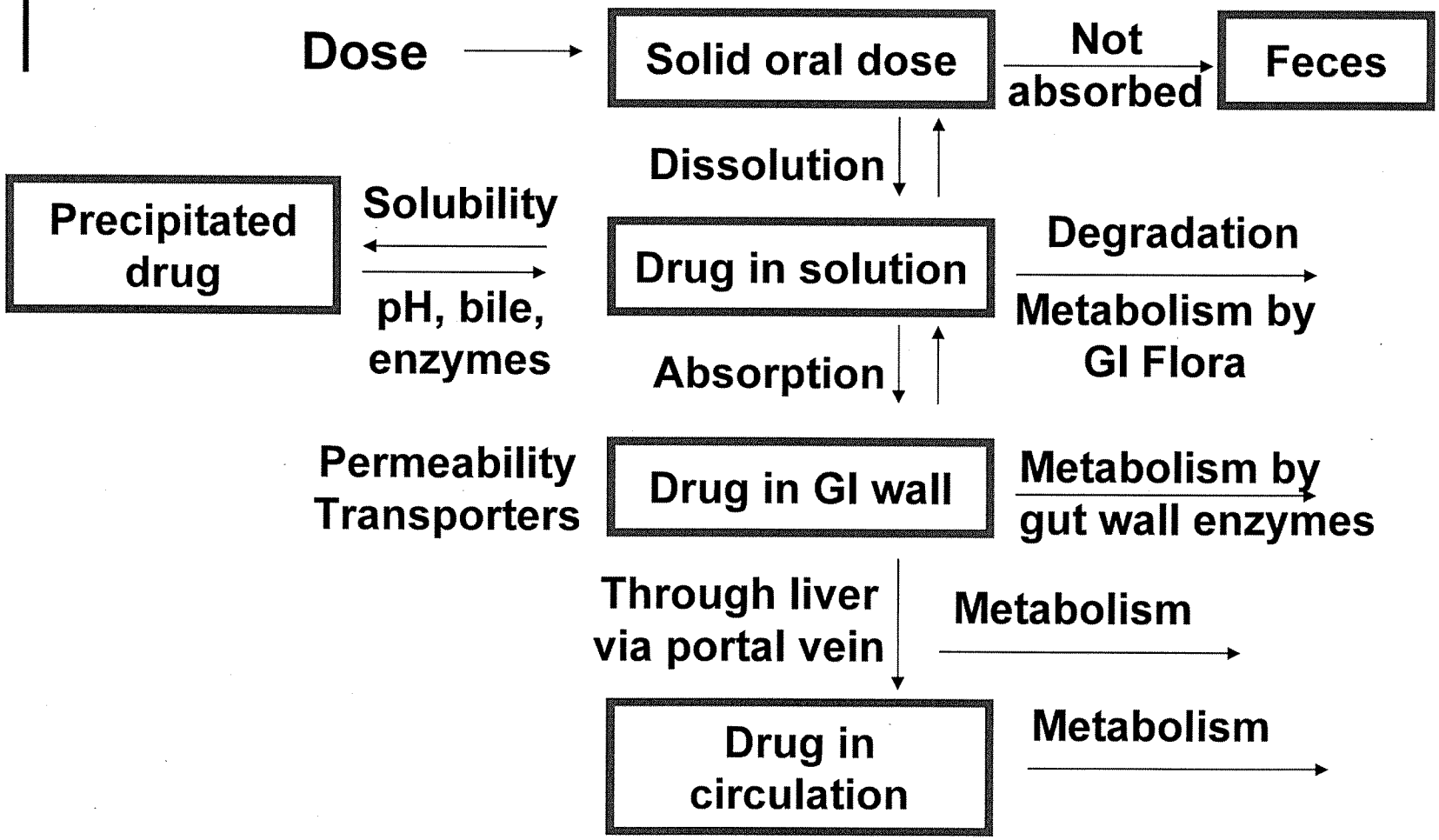
Review of Drug Absorption

Simple Scheme of Oral Drug Absorption



More Complicated Model of Oral Absorption Process



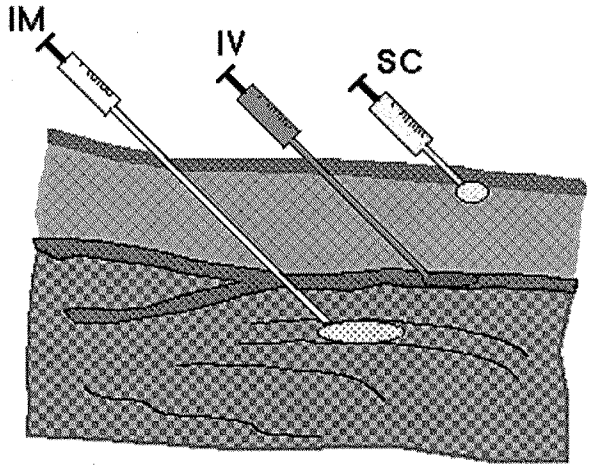
BIOPHARMACEUTICS

Biopharmaceutics is the study of the interrelationship of the physicochemical properties of the active pharmaceutical ingredient, API, and the drug product on the *in vivo* performance of the drug. Biopharmaceutics also considers the formulation of the drug product including excipients, the method of manufacturing, and the route of drug administration.

BIOPHARMACEUTICS – Using BA and BE Information

In terms of regulatory **product quality attributes**, results from BE studies and certain BA studies may be viewed as "product quality performance specifications". A specific **regulatory** challenge is to validate the methods used to assess the results from these studies to assure that the BA and BE data generated relate in a well-defined and meaningful way to safety and efficacy of the drug product.

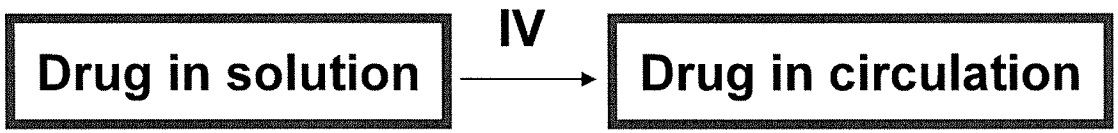
Administration Routes Other Than Oral



- Intravenous Injection
- Topical Adminstration
  - ophthalmic
    - Fast release
    - Sustained release
  - Dermal
    - Gels, creams, ointments
- Intramuscular or subcutaneous injection
  - Fast release
  - Sustained release
- Inhalation
- Nasal
- Rectal
- Buccal or sublingual

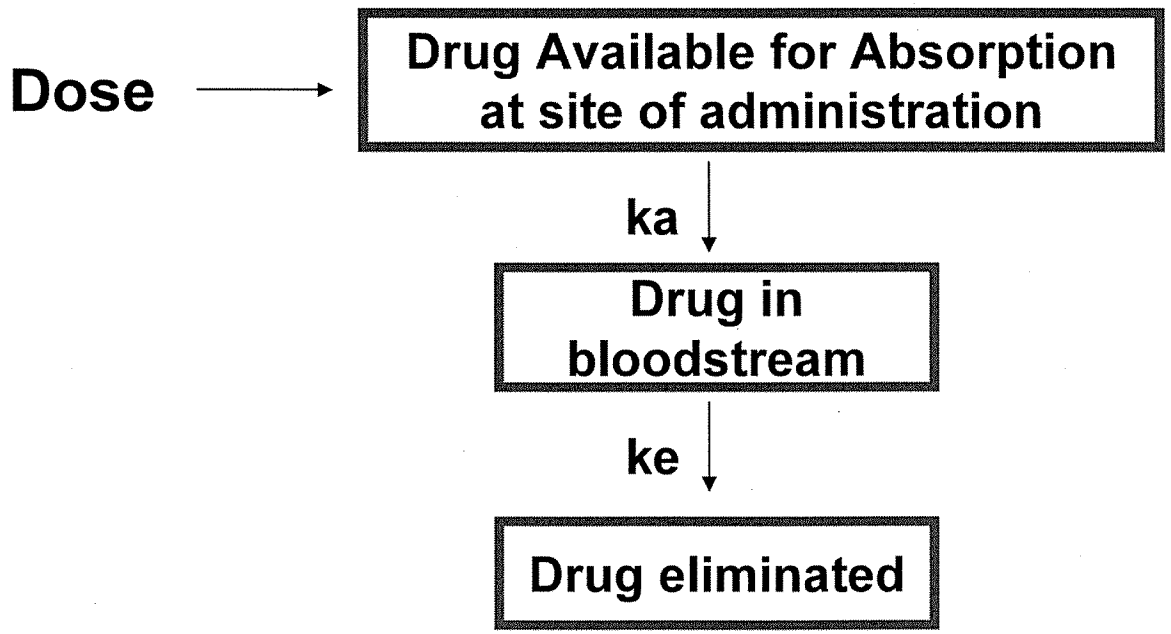
Intravascular (intravenous) dosing-

no absorption process involved



Extravascular (oral, intramuscular, inhalation, other routes)-

absorption process involved, entire dose may not be absorbed



Bioavailability

What is bioavailability?

- **Usually, the "bioavailability" is the fraction of an extravascular dose that gets to the central blood compartment.**
- **Exceptions exist**
  - **Topical dosing (bioavailability is then drug delivered to site of action)**
- **IV doses are 100% bioavailable**
  - **The basis for absolute bioavailability calculations**

Why do we care about BIOAVAILABILITY?

- **The "true dose" is not the drug swallowed; BUT is the drug available to exert its effect.**
  - **Dissolution**
  - **Absorption**
  - **Survive metabolism**
- **May have a drug with very low bioavailability**
  - **Dosage form or drug may not dissolve readily**
  - **Drug may not be readily pass across biological membranes (i.e. be absorbed)**
  - **Drug may be extensively metabolized during absorption process (first-pass, gut wall, liver)**
- **Important component of overall variability**
  - **Variable bioavailability may produce variable exposures**

How is bioavailability measured?

- **Well-controlled animal study or well-controlled clinical trial in humans.**
  - **Measure exposures and time course of exposures**
    - **Area under the concentration vs time curve (AUC)**
    - **Maximum concentration (Cmax)**
    - **Time of Cmax (tmax)**
- **Control variables that might affect outcome**
  - **Healthy "normal" subjects**
  - **Control food and fluid intake**
  - **May control activity, dosing time of day, etc. depending on specific drug or formulation characteristics**

How is bioavailability measured?

- **Usually single dose study**
  - **Usually a crossover study to minimize variability and increase power**
  - **Frequent blood sampling for pharmacokinetic analysis**
- **May measure "relative bioavailability" or "absolute bioavailability"**
  - **"Absolute bioavailability" compares an extravascular formulation to an IV formulation**
  - **"Relative bioavailability" compares 2 extravascular formulations**

Bioavailability Calculations are based on solving these simultaneous eqns.

Systemic clearance:

$$CL(iv) = \frac{Dose(iv)}{AUC(iv)}$$

Apparent (oral) clearance:

$$CL(oral) = \frac{CL(iv)}{F} = \frac{Dose(oral)}{AUC(oral)}$$

How to Calculate Bioavailability

Set CL(iv) equivalent:

$$CL(iv) = \frac{Dose(iv)}{AUC(iv)} = \frac{Dose(oral) \bullet F}{AUC(oral)}$$

Solving for F:

$$F = \frac{Dose(iv) \bullet AUC(oral)}{Dose(oral) \bullet AUC(iv)}$$

Example Bioavailability Calculation

Oral dose: 50 mg

IV dose: 10 mg

Oral AUC(0-∞): 3540 ng • hr/mL

IV AUC(0-∞): 980 ng • hr/mL

Solving for F:

$$F = \frac{\text{Dose(iv)} \bullet \text{AUC(oral)}}{\text{Dose(oral)} \bullet \text{AUC(iv)}} = \frac{10 \bullet 3540}{50 \bullet 980} = 0.72$$

Specialized Oral Dosage Forms and Rate of Absorption

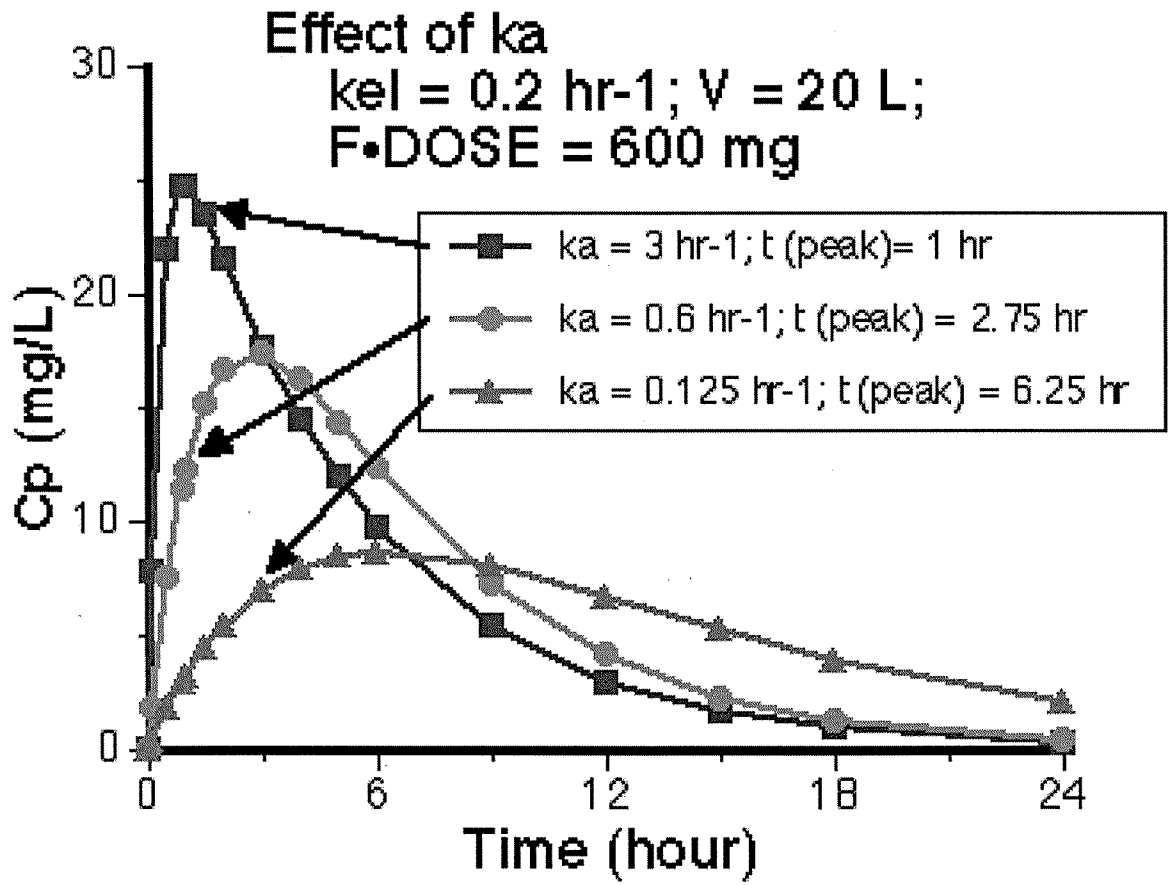
Rate versus Extent of Absorption

- **Extent of absorption is reflected by AUC**
  - **Measured through infinity until *all* drug absorbed**
- **Rate of absorption, ka, is reflected by Tmax**
  - **Presumed to be insensitive to extent of absorption**
  - **Tmax is often an insensitive measure of ka**
- **Both Rate and Extent of absorption affect Cmax**
  - **Rate (R) and Extent (E) may be independent**
  - **Leads to 4 possible relative scenarios:**
    - **(R) Rapid, (E) Complete Absorption yields a short Tmax, high Cmax, high AUC**
    - **(R) Rapid, (E) incomplete absorption yields a short Tmax, low Cmax, low AUC**
    - **(R) Slow, (E) complete absorption yields a long Tmax, high Cmax, high AUC**
    - **(R) Slow, (E) incomplete absorption yields a long Tmax, low Cmax, low AUC**

Examples of these scenarios … varying degrees possible

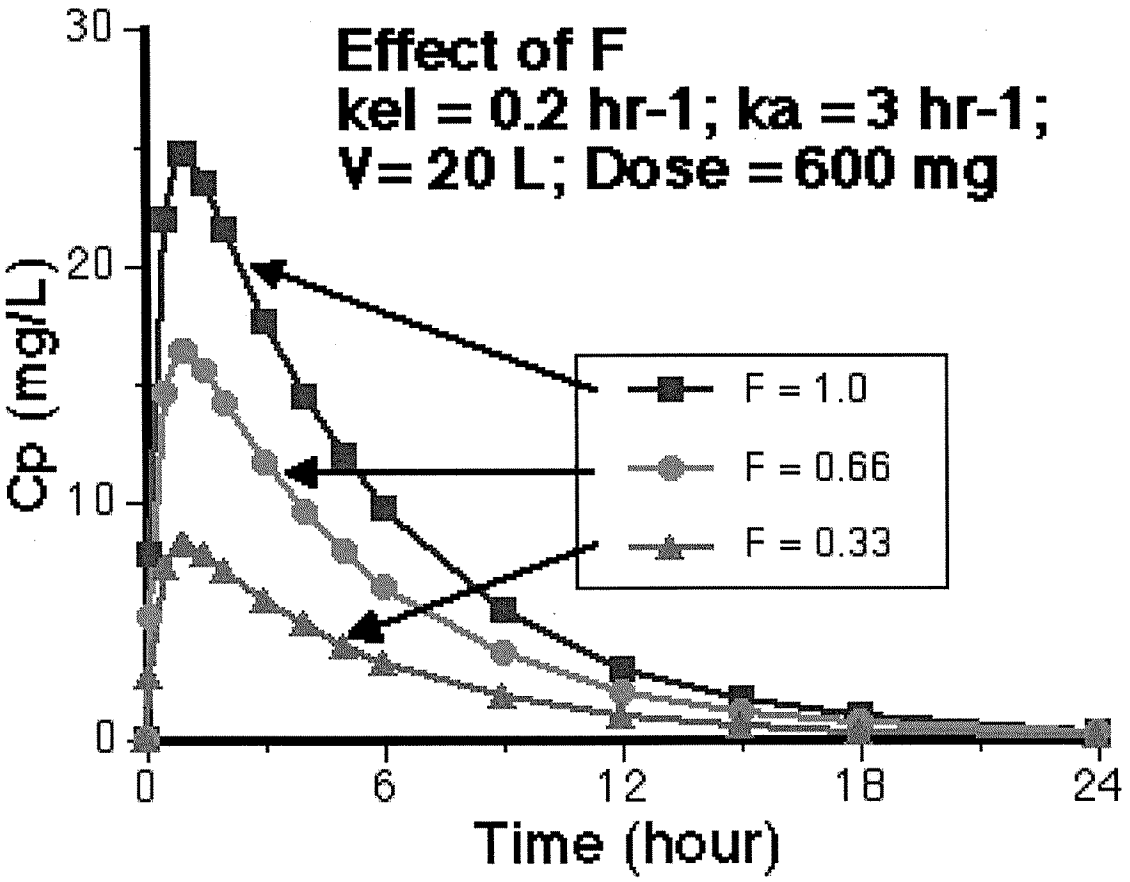
- **Theoretical Examples of the 4 scenarios:**
  - **Rapid, complete absorption**
    - **High solubility, high permeability, not metabolized**
  - **Rapid, incomplete absorption**
    - **High solubility, high permeability, extensively metabolized**
  - **Slow, complete absorption**
    - **Low solubility or permeability, not metabolized**
  - **Slow, incomplete absorption**
    - **Low solubility or permeability, extensively metabolized**

Rate versus Extent of Absorption (Rate Varies)



Courtesy of David Bourne, http://www.boomer.org/c/p4/c08/c0804.html

Rate versus Extent of Absorption (Extent Varies)



Courtesy of David Bourne, http://www.boomer.org/c/p4/c08/c0804.html

Rationale for Specialized Oral Dosage Forms – Using Bioavailability to Advantage

- **Modified release dosage forms:**
  - **any formulation from which release of the drug is delayed or from which released is slower (sustained or controlled) compared to an "immediate release" dosage form.**
- **Developed based on the rationale there is a relationship between exposure to drug or metabolite and the pharmacological or toxicological drug response.**
- **Aim is to achieve a similar total exposure (AUC) as the immediate release form.**
  - **A different nominal dose may be given to achieve this, since F is often different.**

Specialized Oral Dosage Form Characteristics

- Immediate release
  - Solution
    - Absorption usually rapid and extensive
    - May precipitate to form a fine suspension
    - If poorly water soluble, may be mixed with alcohol or glycerol
  - Suspension
    - High surface area maximizes potential for rapid dissolution
    - Particles must disperse, cannot clump
    - Absorption rate often correlates with particle size

Oral Dosage Form Characteristics (continued)

- Immediate release (continued)
  - Capsule
    - Hard shell encloses drug material
    - Shell disintegrates *in vivo* to allow release of contents
    - Water insoluble drugs need dispersing agents to ensure mixing
  - Tablet
    - Very common dosage form
    - Tablet must disintegrate before it can dissolve and release drug
    - Absorption may be dissolution rate-limited

Oral Dosage Form Characteristics (continued)

- **Sustained or Controlled Release**
  - Release drug slowly over time
  - Slow *rate* of absorption, may have extensive *extent* of absorption
  - Relatively flat concentration-time profile (reduce rate of absorption, maintain extent of absorption)
  - Examples are timed release coated pellets, matrices, erodable tablets, and osmotic pumps
- **Delayed Release**
  - Release drug quickly after a delay
  - Time lag before absorption starts, but can have rapid and extensive absorption after release
  - May be used to protect acid-labile drugs from stomach acid (example enteric coated pellets)

Studies to Evaluate Modified Release Dosage Forms

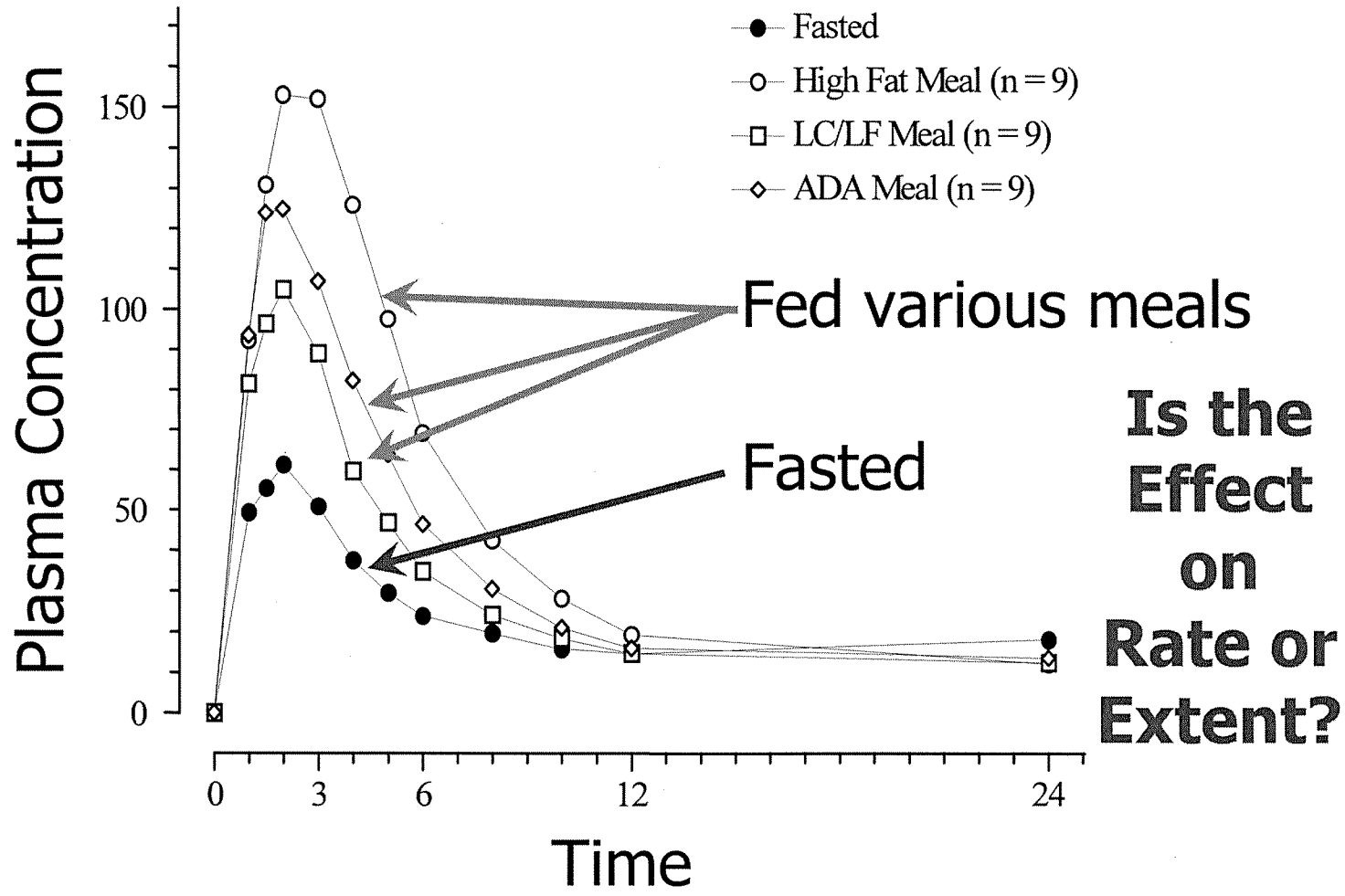
- **Purpose is to characterize:**
  - Rate and extent of absorption
  - Fluctuations in drug concentrations
  - Variability in pharmacokinetics arising from the drug formulation
  - Dose proportionality
  - Factors influencing the performance of the modified drug formulation
  - Risk of unexpected release characteristics (e.g. dose dumping, food effects)
- **Immediate release product of same drug may serve as the reference product.**
  - Once daily CR versus 4-times daily IR -- goal is to achieve the same AUC

Factors That Affect Bioavailability

Food Effects on Bioavailability

- Food may increase, decrease, or have no effect on the rate and/or the extent of absorption
  - May affect rate and extent independently.
  - Food Effects GI motility and also can increase solubilization of drugs
  - Change may depend on content of meal
- Food may mitigate nausea
  - Vomiting tends to decrease bioavailability
- Time of dose with respect to food

Example
Food increasing bioavailability



Drug Metab. Rev. 34(Supplement 1):145 (2002).

Gut Physiology Related to Absorption

- Bilayer structure of cell membranes
  - Drugs too lipophilic won't dissolve
  - Drugs too hydrophilic won't transverse lipid outer layer of cell
- Thickness and blood supply of membranes
- GI transit time
  - acetaminophen is a useful probe drug to assess GI transit
- pH environment
  - Very acidic in stomach
  - Neutral to basic in small intestine
    - Depending on pKa, drug may be charged or uncharged in different regions
- Metabolic activity, especially induction or inhibition
- Surface area of membrane = absorption surface
  - Stomach low surface area
  - Small intestine high surface area

GI Physiology and Drug Absorption

	pH	Membrane	Blood Supply	Surface Area	Transit Time	By-pass liver
BUCCAL	approx 7	thin	Good, fast absorption with low dose	small	Short unless controlled	yes
ESOPHAGUS	5 - 6	Very thick, no absorption	-	small	short	-
STOMACH	1 - 3 decomposition, weak acid unionized	normal	good	small	30 - 40 minutes, reduced absorption	no
DUODENUM	6 - 6.5 bile duct, surfactant properties	normal	good	very large	very short (6" long), window effect	no
SMALL INTESTINE	7 - 8	normal	good	very large 10 - 14 ft, 80 cm ^{2} /cm	about 3 hours	no
LARGE INTESTINE	5.5 - 7	-	good	not very large 4 - 5 ft	long, up to 24 hr	lower colon, rectum yes

Courtesy of David Bourne, http://www.boomer.org/c/p1/Ch11/Ch1103.html

Methods used to Assess Gastric Emptying Rate

Gastric emptying studied >160 years many techniques:

- Radiologic
  - liquid barium sulphate
  - enteric-coated barium granules
  - "barium burger"
  - radioopaque spheres
- Intubation-aspiration
  - saline load test
  - serial intubation and aspiration
  - multiple sampling multilumen tube perfusion and aspiration
- Radioisotopic
  - solid phase emptying
  - liquid phase emptying
- Ultrasound
- Absorption kinetics of orally-administered solutes
  - Acetaminophen ethanol glucose
- Ferromagnetic tracer
- Epigastric impedance
- Applied potential tomography

pH-Dependent Effects

- **pH-Partition Theory**
  - **Un-ionized drug is absorbed through membranes**
  - **Charges species don't get through easily**
- **Depends on molecule's pKa**
  - **Innate characteristic of the drug molecule**
  - **pH at which half the molecules are charged, half are neutral**
- **For acids, a pH below the pKa enhances absorption**
- **For bases, a pH above the pKa to enhances absorption**

Other Factors Affecting Bioavailability

- **Instability of the drug itself in the GI tract can have impact on F**
  - **Due to chemical instability in acidic environment**
  - **Due to extent of metabolism via enzymes in gut**
- **Drugs that affect the following may result in drug interactions and affect bioavailability of concomitant meds**
  - **Enzyme activity in the gut or liver**
    - **Induced enzymes will lower bioavailability**
    - **Inhibited enzymes will increase bioavailability**
  - **Gastric motility**
  - **Gastric pH**

Bioequivalence

Historical Perspective on BE testing

- BE was established in 60-70's due to classic therapeutic equivalence problems
- Classical ANOVA and paired time-point sample testing – replicate data statistical testing
- Evolved testing methods for specific BE parameters of Cmax, Tmax, and AUC
  - 75/75 Rule (75% subjects within 75% of reference)
  - Log Transformations and CI Testing
  - Average Bioequivalence
    - Mean response assessed

State of the Art

  - Population Bioequivalence
    - Mean and variance assessed
  - Individual Bioequivalence
    - Replicate Study Design (formulation against itself)

Goal of BA and BE testing

- **BE is defined as:**
  - **"the absence of a significant difference in the rate and extent to which the active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives becomes available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study."**

FDA Guidance for Industry:
Bioavailability and Bioequivalence Studies for Orally Administered Drug Products — General Considerations

Broad Goals of BA and BE testing

- Both BA and BE evaluations are important elements of drug development and INDs, NDAs, and ANDAs.
- BA studies
  - Characterize exposure and PK profile of drug
  - Serve as benchmark for subsequent BE studies
- BE studies
  - Establish that a new formulation has therapeutic equivalence in the rate and extent of absorption to the reference drug product.
  - Important for linking the commercial drug product to clinical trial material at time of NDA
  - Important for post-approval changes in the marketed drug formulation

Design of BE Studies

- **Design is key as these studies are critical in regulatory applications.**
  - **Sample size must be appropriate and based on intrasubject variability for the primary measures of interest (Cmax and AUC).**
  - **Careful selection of the treatments used in study:**
    - **Select "test product" so it best represents commercial drug manufacturing lots at the correct scale, manufacturing site, and unit formula.**
    - **Select "reference product" in order to successfully link previous formulations used in development.**
    - **Maintain "biosamples" (house sample of drug products tested) due to 1980's generic drug industry scandal**

BE Studies for Generic Drugs

- BE studies are a critical component of ANDA submissions. The purpose of these studies is to demonstrate BE between a pharmaceutically equivalent generic drug product and the corresponding reference listed drug (marketed drug)
- Typically, efficacy studies are not required for ANDAs, so the BE study is key to showing therapeutic equivalence.

FDA Guidance for Industry:
Bioavailability and Bioequivalence Studies for Orally Administered Drug Products — General Considerations

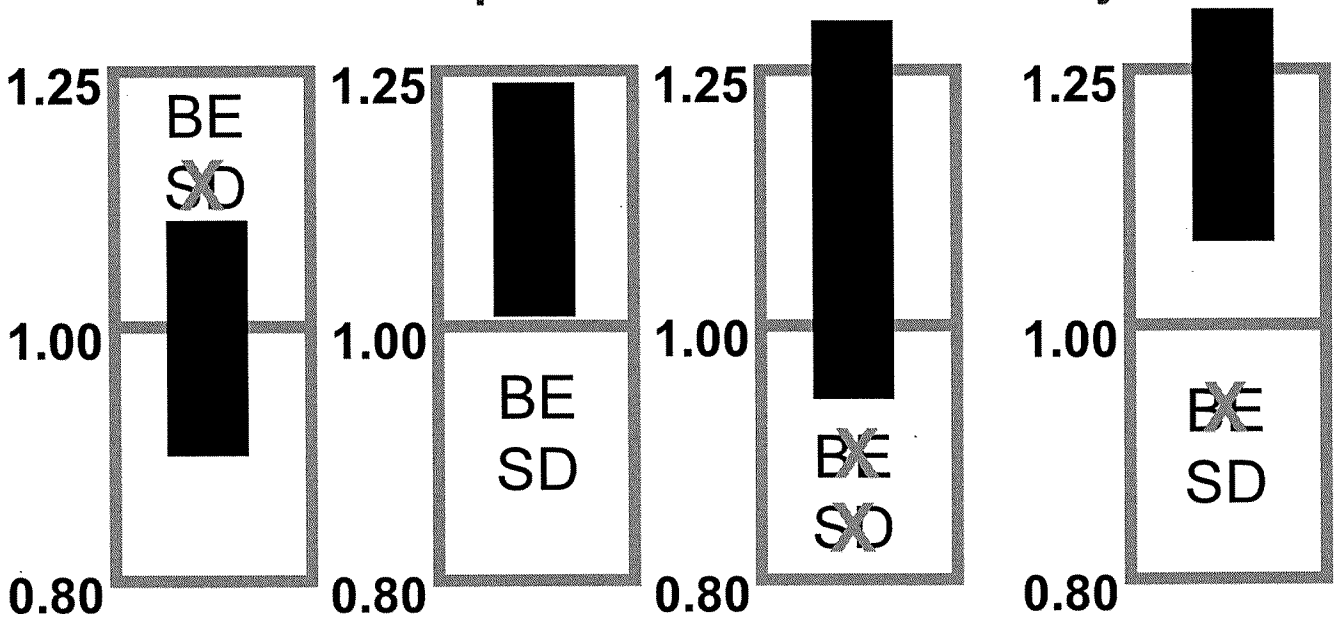
Role of Bioequivalency for Innovator Drugs

- **The ultimate goal for all marketed products is to be safe and efficacious**
  - **Efficacy based on pivotal clinical trials utilizing defined dosage form(s) manufactured by defined process**
  - **Safety obtained from all clinical trials**
- **Bioequivalence studies assess in vivo impact of changes to the dosage form/process after pivotal studies commence to ensure product on the market is comparable to that upon which the efficacy is based**

BE Confidence Intervals

- **For Bioequivalence, the 90% confidence interval of F′ must fall between 0.8 and 1.25**

BE = bioequivalent SD = Statistically Different



Current FDA Guidance Documents on Bioequivalence

- Potassium Chloride Modified-Release Tablets and Capsules: In Vivo Bioequivalence and In Vitro Dissolution Testing [10/26/2005]
- Clozapine Tablets: In Vivo Bioequivalence and In Vitro Dissolution Testing [6/20/2005]
- **Handling and Retention of Bioavailability and Bioequivalence Testing Samples** [05/26/2004]
- Bioavailability and Bioequivalence Studies for Nasal Aerosols and Nasal Sprays for Local Action - 2nd Draft [04/03/2003]
- **Bioavailability and Bioequivalence Studies for Orally Administered Drug Products – General Considerations** [03/19/2003]
- **Food-Effect Bioavailability and Fed Bioequivalence Studies** [01/31/2003]
- Liposome Drug Products: Chemistry, Manufacturing, and Controls; Human Pharmacokinetics and Bioavailability; and Labeling Documentation [08/21/2002]
- Levothyroxine Sodium Tablets -- In Vivo Pharmacokinetic and Bioavailability Studies and In Vitro Dissolution Testing [03/08/2001]
- **Statistical Approaches to Establishing Bioequivalence** [02/02/2001]
- **Waiver of In Vivo Bioavailability and Bioequivalence Studies for Immediate Release Solid Oral Dosage Forms Based on a Biopharmaceutics Classification System** [08/31/2000]
- SUPAC-MR: Modified Release Solid Oral Dosage Forms: Scale-Up and Postapproval Changes: Chemistry, Manufacturing, and Controls, In Vitro Dissolution Testing, and In Vivo Bioequivalence Documentation [10/06/1997]
- SUPAC-SS - Nonsterile Semisolid Dosage Forms; Scale-Up and Postapproval Changes: Chemistry, Manufacturing, and Controls; In Vitro Release Testing and In Vivo Bioequivalence Documentation [06/13/1997]
- SUPAC-IR Immediate-Release Solid Oral Dosage Forms: Scale-Up and Post-Approval Changes: Chemistry, Manufacturing and Controls, In Vitro Dissolution Testing, and In Vivo Bioequivalence Documentation [11/30/1995]
- Letter on incomplete Abbreviated Applications, Convictions Under GDEA, Multiple Supplements, Annual Reports for Bulk Antibiotics, Batch Size for Transdermal Drugs, Bioequivalence Protocols, Research, Deviations from OGD Policy [04/08/1994]
- Letter to regulated industry notifying interested parties about important detailed information regarding labeling, scale-up, packaging, minor/major amendment criteria, and bioequivalence requirements [08/04/1993]
- Cholestyramine Powder In Vitro Bioequivalence [7/15/1993]
- Letter on the provision of new information pertaining to new bioequivalence guidelines and refuse-tofile letters [07/01/1992]